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Optimisation of biofilm formation by *Cryptococcus neoformans* H99

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Background: *Cryptococcus neoformans* is one of the leading cause of death in immunocompromised individuals, particularly ones diagnosed with acquired immune deficiency syndrome (AIDS). This fungus has the ability to form biofilm which gives higher survivability advantages in harsh environmental stress and confers resistance to antifungal drugs. Different environmental conditions such as type of nutrient medium, cell density, and incubation duration, affect the way biofilm is formed. The understanding of these optimised conditions allows better platform for future *C. neoformans* biofilm studies.

Methods: *C. neoformans* H99 was grown in minimal media and RPMI-1640. For each medium, the cultures were seeded with three different concentrations (103, 105, and 107 cells/ml) for 48 or 72 hours and incubated at 37 °C. Resulting growth was examined using an inverted microscope (Nikon Eclipse T*i*, Japan) at 400× magnification. The cells were examined under the microscope again after the washing with PBS (with 0.05% Tween 20) after which the cells were considered as true biofilms.

Results: In this study, biofilm formation appeared to be optimised in RPMI-1640 medium, 105 cells/ml concentration and 48 hours of incubation duration. The biofilm formed in RPMI medium at initial inoculum concentration of 103 cells/ml for both 48 and 72 hours incubation period was observed to be highly packed and localised. However, at 107 cells/ml, the cryptococcal cells appeared to be loosely packed but well distributed on the solid surface.

Conclusion: This study reports the optimised condition in addition to the current understanding of conditions for *in vitro* formation of biofilm of *C. neoformans*. This knowledge can be applied in the assay setting of antimicrobial susceptibility testing targeting the biofilm of this fungus. Based on our microscopic observation, biofilm of *C. neoformans* is best formed after 48 hours incubation in RPMI-1640 with initial cell concentration of 105 cells/ml.

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Establishing role of CD4+ cell count in predicting *Penicillium marneffei* infection among HIV positive patient in infectious disease centre, Malaysia

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Background: *Penicillium marneffei* infection (penicilliosis) is endemic particularly in Southeast Asia and has emerged as an AIDS-defining illness. To date, there are minimal reports regarding penicilliosis amongst HIV-positive patient from Malaysia. The objective of this study is to establish the role of CD4+ cell count in administration of early treatment and prophylaxis.

Methods: A retrospective study was conducted on *P. marneffei* isolates collected from Microbiology Unit, Hospital Sg. Buloh, Selangor, Malaysiafrom December 2010 to December 2011. The CD4+ cell count of all patients with penicilliosis were collected, along with clinical history.

Results: The CD4+ cell count level was divided into four categories: less than 10 cells/μL, 11-50 cells/μL, 51-100 cells/μL and more than 100 cells/μL. Majority of the patients (43%) contracted penicilliosis only when CD4+ cell count level falls below 10 cells/μL. Another 33% of patients developed penicilliosis when CD4+ cell count level is between 11 to 50 cells/μL. The remaining 7% and 10% of patients acquired penicilliosis when CD4+ cell count level are 51-100 cells/μL and more than 100 cells/μL, respectively.

In addition to CD4+ cell count, other clinical parameters were documented. There was 70 isolates of *P. marneffei* from 30 HIV positive patients, isolated from blood (87%), skin (20%), followed by bone marrow and body fluid (3% respectively). Thirteen (44%) of those patients develop penicilliosis within a year of being diagnosed with HIV. The commonest presenting complaint was fever (60%), followed by respiratory (50%), gastrointestinal (44%) and dermatological manifestations (20%). Tuberculosis (30%) is the other identified predisposing factor. The most frequent treatment administered was amphotericin B followed by itraconazole (37%), and amphotericin B followed by fluconazole (27%).

Conclusion: The CD4+ cell count has the potential to be a useful predictive tool for penicilliosis in HIV positive patient. Prophylaxis should be considered once these group of patients' CD4+ cell count falls below 50 cells/μL with presence of suspicious clinical manifestation. This should be performed even prior to the presence of any microbiological evidence of penicilliosis.

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